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## Application of gene expression analyses to studies of general flowering

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**Abstract** General flowering is a community-level mass flowering observed at irregular intervals of less than one year to 10 years in South-East Asian tropical rainforests. Several hypotheses have been proposed to explain the mechanism of general flowering, but it is still not clear how many triggers can induce general flowering and whether sensitivities to the trigger(s) are variable in different populations. To answer these questions, we review a method integrating gene expression data into environmental and endogenous data. Our study suggests that the function of the *FLOWERING LOCUS T (FT)* gene is conserved as a floral activator in dipterocarps, which makes it possible to detect the precise timing of flower induction from the expression of the *FT* homologues in dipterocarps. Combined with the environmental and endogenous data during flower induction, this method has the potential to become a powerful tool to identify the trigger(s) of general flowering. We also discuss the future application of this method to well-planned seed collection strategies for forest restoration.

**Keywords** Dipterocarpaceae, General flowering, Flowering, Gene expression, *FLOWERING LOCUS T*

### Introduction

General flowering is a community-level mass flowering observed at irregular intervals of less than one year to 10 years in South-East Asian tropical rainforests, where Dipterocarpaceae is a dominant family (Ashton et al. 1988; Appanah 1993; Sakai et al. 1999; Yasuda et al. 1999; Sakai

2002). Diverse plant species, including the majority of the species of Dipterocarpaceae, flower synchronously during general flowering (Sakai et al. 1999). Because of these unique characters, scientists have been attracted to this mysterious flowering phenomenon and have proposed several hypotheses to explain how general flowering is induced. The proposed factors that induce general flowering can be classified into two major categories: environmental and endogenous factors. Through the comparisons between meteorological data and the patterns of general flowering, prolonged drought (Medway 1972; Appanah 1985; Sakai et al. 2006; Brearley et al. 2007), increased sunshine hours due to less cloudy conditions (Wright and van Schaik 1994), increases or decreases in mean air temperature (Appanah 1985), and falls in minimum air temperature (Ashton et al. 1988; Yasuda et al. 1999) have been proposed as candidate environmental triggers. Furthermore, as a candidate endogenous trigger, the accumulation of phosphorus has been suggested based on the observation of the phosphorus level before and after general flowering (Ichie and Nakagawa 2013). Some recent studies from several sites obtained results supporting the drought hypothesis (Sakai et al. 2006; Brearley et al. 2007; Kobayashi et al. 2013; Numata et al. 2013). However, because drought is more frequently observed than general flowering in Peninsular Malaysia (Numata et al. 2013), it is still unclear whether drought is the sole trigger of general flowering. Furthermore, additional studies are needed to test whether a single threshold of drought level can be applied to all the populations as a trigger of general flowering.

Recent studies using a model plant, *Arabidopsis thaliana*, revealed that flowering is regulated by multiple environmental and endogenous factors. As environmental factors, day length (photoperiod), prolonged cold temperature in winter (vernalization), light quality, and ambient temperature are known to regulate flowering in *A. thaliana* (Amasino 2010; Fornara et al. 2010). Furthermore, a plant hormone, gibberellin, the age of the plant, and carbohydrate concentration are utilized as endogenous information to induce flowering (Amasino 2010; Fornara et al. 2010; Wahl et al. 2013). In other plant species, flowering is also regulated by multiple factors (Amasino 2010; Shrestha et al. 2014). Therefore, it is possible that multiple factors can induce general flowering in dipterocarps. Furthermore, studies in *A. thaliana* have shown that sensitivities to the flowering cues are genetically differentiated in individuals from different populations (Lempe et al. 2005), suggesting that sensitivities to the trigger(s) of general flowering could also differ among populations in dipterocarps. The possibilities of multiple triggers and their variable sensitivities among populations make it difficult to identify the trigger(s) of general flowering.

One possible solution is to detect more precisely the timing of the induction of general flowering in each population and to test what kinds of environmental and endogenous changes occur during the induction period. To achieve this, we are developing a method based on gene expression. In this review, we introduce our current approach and future goals.

#### **Detection of precise timing of flower induction by gene expression**

It is difficult from morphological data to detect the precise timing of receiving flowering triggers, that is flower induction, because a substantial delay exists by the time morphological flower development is observed after flower induction (Schmid et al. 2003). This is one of the obstacles to identifying the trigger(s) of general flowering from morphological phenology data. To overcome this problem, application of the knowledge of flowering gene networks and gene expression analysis is a useful method (Kobayashi and Shimizu 2011; Shimizu et al. 2011).

While flowering in *A. thaliana* is regulated by multiple environmental and endogenous

factors, these flowering signals converge into a few flowering genes called floral pathway integrators (Amasino 2010; Fornara et al. 2010; Shrestha et al. 2014). Among them, *FLOWERING LOCUS T (FT)* is a key floral activator (Kardailsky et al. 1999; Kobayashi et al. 1999). The homologues of the *FT* gene have been identified in monocots, such as rice and temperate cereals, as well as dicot species, suggesting that this gene is well conserved in angiosperms (Amasino 2010; Shrestha et al. 2014). The expression of *FT* is upregulated just after the flower induction when morphological changes are not observed (Schmid et al. 2003). Therefore, it is thought that the expression of the *FT* gene can be used as an indicator to detect flower induction (Kobayashi and Shimizu 2011). Using this idea in a mass flowering species, *Fagus crenata*, in Japan, Miyazaki et al. (2014) detected the timing of flower induction from the expression of the homologue of *FT* and showed higher nitrogen levels during the flower induction periods only in the years when flower induction occurs. Furthermore, through experimentally manipulating the nitrogen levels, it was shown that nitrogen is one of the key flowering regulators in *Fagus crenata*. Thus, through applying this method, we would expect also to be able to detect the precise timing of the induction of general flowering from the expression levels of the *FT* homologues in dipterocarps and to identify the trigger(s) of general flowering.

#### **Identification and characterization of the homologue in *Shorea beccariana***

To apply the method discussed above, first, it is necessary to identify and characterize the *FT* homologues in dipterocarps. During the general flowering event in 2009, we conducted a whole-genome transcriptome analysis with a next-generation sequencer, Roche 454, by using samples collected from a species of Dipterocarpaceae, *Shorea beccariana*, in Lambir Hills National Park, Sarawak, Malaysia (Kobayashi et al. 2013). The transcriptome analysis detected expression changes in 1,128 genes during general flowering. Among the differentially expressed genes, a homologue of the *FT* gene was found and named *SbFT*. Consistent with the drought hypothesis, the expression analysis using real-time PCR showed that the expression of *SbFT* was upregulated after prolonged drought before flowering when flower buds were still not observed morphologically. However, we note that it was still not clear exactly when the upregulation of *SbFT* started because of our long sampling intervals. Then, to test the function of *SbFT*, we used transgenic *A. thaliana* plants overexpressing this gene. The transgenic plants showed early flowering, suggesting that *SbFT* acts as a floral activator. These results indicate that the *FT* functions are conserved in dipterocarps. Thus, the expression of the *FT* homologues in dipterocarps can be used as a marker to detect the timing of induction of general flowering.

#### **Identification of trigger(s) of general flowering by the expression of the *FT* homologues**

Regular sampling is essential for detecting the precise timing of induction of general flowering from the gene expression. Although frequent sampling is difficult, regularly and frequently collected expression data will produce outstanding results (Aikawa et al. 2010, Nagano et al. 2012). In addition to the regularly collected expression data, measurements of meteorological and endogenous data are required in this method. In Lambir Hills National Park, many environmental variables, such as rainfall, temperature, and solar radiation, have been measured over a long term (Kume et al. 2011). This is of great advantage for studying the trigger(s) of general flowering related to the gene expression at this study site. Long-term observations of meteorological and gene expression data, including multiple general flowering events, will answer the question of

whether or not drought is the sole trigger of general flowering. Furthermore, if the studies using this genetic method are conducted at several study sites together with Lambir Hills National Park, the question of whether or not sensitivities to the trigger(s) are different among populations will also be answered.

### **Application of the expression approach to restoration strategies of tropical rainforests**

To ensure proper management and restoration of tropical rainforests, adequate seed collection is required. For this purpose, we need to know when general flowering occurs because general flowering is an important source of seeds for forest restoration. To predict the occurrence of general flowering prior to flower development, measuring the expression of the *FT* homologues would also be a good method because *FT* expression precedes morphological flower development (Kobayashi et al. 2013). This would give us enough time to prepare for seed collection before general flowering. Thus, we hope that this method will be applicable to forest restoration as well as to the identification of the trigger(s) of general flowering.

### **Conclusion**

In this review, we introduced a method of integrating gene expression data with environmental and endogenous data to identify trigger(s) of general flowering. Our recent work suggested that the *FT* function as a floral activator is conserved in dipterocarps, which makes it possible to detect the precise timing of flower induction from the expression of the *FT* homologues in dipterocarps. Combining the environmental and endogenous data during flower induction, this method could become a powerful tool for identifying the trigger(s) of general flowering. Moreover, we hope that this method will be applicable to well-planned seed collection strategies for forest restoration, because the expression of *FT* homologues can tell us whether or not general flowering will occur prior to the appearance of flowers. However, to conduct this method, environmental and endogenous data as well as expression data are required through regular sampling. Furthermore, to understand the variable sensitivities to the trigger(s) among populations, studies using this method need to be conducted at multiple study sites. To achieve this, further collaborations of researchers of different disciplines and at different study sites between Sarawak and Japan would be essential.

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